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Note

Separation and detection of sugars and alditols on thin layer chromatograms

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Abstract

A thin-layer chromatographic method has been developed to separate aldoses and their corresponding alditols. Two ascents of acetonitrile–ethyl acetate–1-propanol–water (85:20:20:15, v/v/v/v) at 20 °C on Whatman K6 plates were used. A dipping detection system using alkaline silver nitrate–sodium thiosulfate was used to detect the alditols in the 500 ng to 1 μg range. The aldoses and their corresponding alditols were D-glucose, D-glucitol, D-mannose, D-mannitol, D-galactose, galactitol, D-xylose, xylitol, D-ribose, ribitol, D-arabinose, D-arabinitol, maltose, and maltitol.
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One of the more difficult chromatographic separations of carbohydrates is the separation of similarly structured monosaccharides, such as D-glucose, D-mannose, and D-galactose, and similar disaccharides such as cellobiose and lactose. These carbohydrates have been successfully separated by thin-layer chromatography (TLC), using a relatively simple solvent of acetonitrile–water (85:15, v/v) [1]. Another difficult separation occurs between aldoses and their corresponding alditols. Paper chromatography has been used to separate aldoses and their alditols [2,3], but it is relatively slow (15–20 h) and requires relatively large amounts of sample (250–500 μg). On the other hand, TLC is relatively fast (1–3 h), requires small amounts of sample (500 ng–1 μg), and is easy to carry out with inexpensive semimicro equipment and materials [4].

The separation of individual alditols from aldoses is important in identifying the reducing-end residue when determining the structure of reducing saccharides [5]. The separation of alditols from their parent aldoses is also necessary in determining the direction of polysaccharide chain-elongation during biosynthesis [6–8]. Further, the use of thin-layer chromatography to separate and quantitate relatively small amounts of radioactively labeled alditols and aldoses by phosphoimaging is very convenient in these determinations. We have, therefore, developed a TLC method for the separation of several common alditols from their corresponding aldoses, and here report a detection method that is sensitive in the μg range.

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1. Experimental

Procedures.—Whatman K6 20×20 cm TLC plates were spotted with various monosaccharides (pentoses and hexoses) and one disaccharide (maltose), and their alditols. The majority of the saccharides were commercial products. Ribitol was prepared by the reduction of D-ribose with $NaBH_4$. The TLC plate was irrigated two times with MeCN–EtOAc–1-propanol–water (85:20:20:15, v/v/v/v) at 20 °C. The solvent was allowed to ascend to the top of the plate and the plate was removed and thoroughly dried between the two ascents. The carbohydrates were visualized on the plate by using an alkaline $AgNO_3$ dipping procedure [9]. The dry TLC plate was dipped into $AgNO_3$–acetone solution for 5 min. The reagent is prepared by the addition of 1 mL of saturated aq. $AgNO_3$ into 200 mL of acetone; water is added dropwise (about 20 drops) with stirring until the $AgNO_3$ completely dissolves. The plate is dried and then dipped into an alkaline MeOH solution, prepared by the addition of 2 mL of 40% w/v NaOH to 200 mL of MeOH, for 30 min. Brown to black spots immediately appear for the carbohydrates. The plate is dried and then rapidly dipped into a 1.5 M $Na_2S_2O_3$ solution containing 0.08 M Na_2SO_3 and 0.25 M $NaHSO_3$. The plate is then washed for 1 min in running water. This gives black spots on a white background.

2. Results and discussion

Seven aldoses and their corresponding alditols have been separated using two ascents of MeCN–EtOAc–1-propanol–water (85:20:20:15, v/v/v/v) at 20 °C on Whatman K6 TLC plates (see Fig. 1). Table 1 gives the migration distances and R_G values for separation of the alditols and their parent aldoses. In all examples, the aldoses were resolved from each other and the alditols were resolved from their corresponding parent aldoses. Exceptions were D-xylose and D-ribose, which were only slightly separated with R_G values of 1.71 and 1.69, respectively, and D-glucitol and D-mannitol, with R_G values of 0.84 and 0.88, respectively. On review of the literature, however, we were unable to find any conventional TLC systems that gave as good a separation of the alditols from their parent aldoses as our system gave. The separation of alditols has been reported by forming complexes with lanthanide cations, followed by separation with triply distilled water on ionic resin chromatosheets [10,11]. This method was able to separate D-glucitol from D-mannitol.

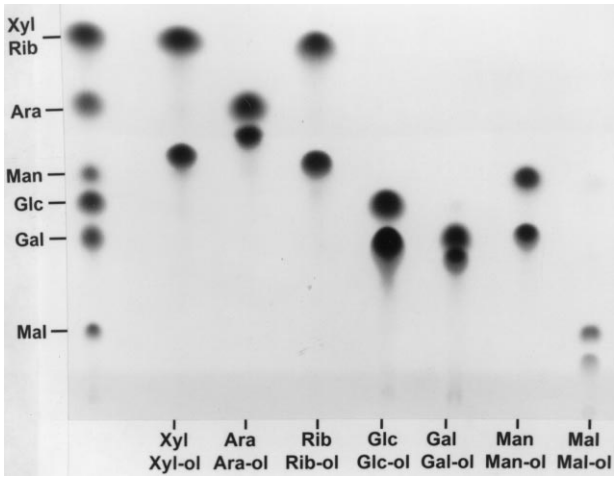

Fig. 1. Separation of aldoses and their alditols on a Whatman K6 TLC plate. D-Xylose (Xyl), xylitol (Xyl-ol), D-arabinose (Ara), D-arabinitol (Ara-ol), D-ribose (Rib), ribitol (Rib-ol), D-glucose (Glc), D-glucitol (Glc-ol), D-galactose (Gal), galactitol (Gal-ol), D-mannose (Man), D-mannitol (Man-ol), maltose (Mal), and malitol (Mal-ol) were separated on the TLC plate with two ascents of acetonitrile–ethyl acetate–propanol–water (85:20:20:15, v/v/v/v) at 20 °C and visualized by the silver nitrate-dip technique.

The TLC carbohydrate detection method of dipping the TLC plate into a methanolic solution of 0.3% (w/v) *N*-(1-napthyl)ethylenediamine and 5% (v/v) sulfuric acid, followed by heating at 120 °C for 10 min is very sensitive for most carbohydrates, detecting amounts down to 50 ng [4,12]. An exception is the alditols, which are only beginning to be detected at around 10 μg (10,000 ng). Presumably this occurs because the method requires the formation of furfural, which is readily formed by the reaction of sulfuric acid with aldoses but not with

Table 1
Migration distances and R_G values for aldoses and their alditols using two ascents of acetonitrile–ethyl acetate–propanol–water (85:20:20:15, v/v/v/v) on Whatman K6 TLC plates at 20 °C

Aldose	DM [a] (cm)	R_G [b]	Alditol	DM [a] (cm)	R_G [b]
D-Xylose	11.7	1.71	Xylitol	8.4	1.23
D-Ribose	11.5	1.69	Ribitol	8.0	1.18
D-Arabinose	9.8	1.43	D-Arabinitol	9.0	1.32
D-Mannose	7.7	1.13	D-Mannitol	6.0	0.88
D-Glucose	6.8	1.00	D-Glucitol	5.7	0.84
D-Galactose	5.8	0.85	Galactitol	5.3	0.78
Maltose	3.1	0.46	Maltitol	2.2	0.32

[a] DM = distance migrated in cm from the origin.
[b] R_G = migration relative to D-glucose.

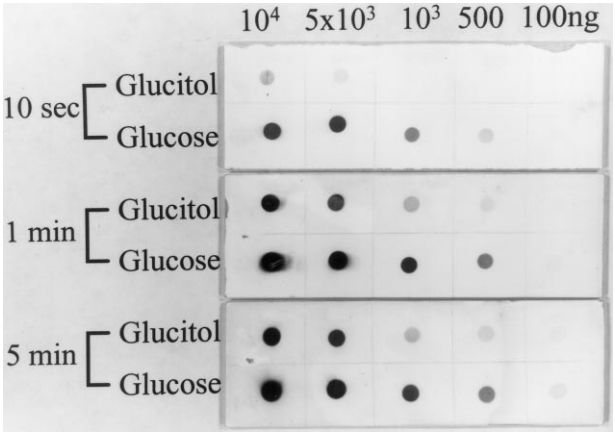

Fig. 2. Sensitivity of the visualization of D-glucose and D-glucitol on a TLC plate by the silver nitrate-dip technique. The TLC plate was spotted with various amounts of D-glucose and D-glucitol (10^4, 5×10^3, 10^3, 500, 100 ng) and visualized by the methods described in the text.

alditols. Detection of alditols by sulfuric acid charring is even less sensitive. The use of silver nitrate dissolved in acetone as a dipping reagent [9], in which the plate is placed into the silver nitrate reagent for 5 min, allows detection of alditols down to 1000 ng (see Fig. 2). While this is still a concentration 20-times higher than the sensitivity for aldoses, it is over 10-times more sensitive than the *N*-(1-napthyl)ethylenediamine–sulfuric acid reagent or the sulfuric acid charring procedure. The time of reaction with silver ion is critical, as seen in Fig. 2. Increasing the time beyond 5 min, however, did not increase the sensitivity further, as the background also increased. The time in the alkaline–methanol reagent of 30 min is also critical for the detection of low amounts of aldoses and alditols. Quantative analysis of these carbohydrates on TLC can be performed by densitometry, using standards, as already reported for the determination of malto- and isomalto-dextrins [4] and O-methylated monosaccharides [12].

Although other detection systems for alditols on TLC have been reported, for example, the use of a dipping reagent of ammonium vanadate with sulfuric acid in acetone [13] and a dipping reagent of thymol with sulfuric acid in 95% ethanol [14], we did not find that these methods were as sensitive for the detection of alditols as were the alkaline silver nitrate dipping reagents.

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